

**APPENDIX B**

1. A method of formulating a composition comprising one or more chemokines for use in a pharmaceutical composition having anti-HIV activity against one or more HIV-1 isolates present in an individual at a given time, the method comprising:

(a) contacting a first aliquot of HIV<sup>+</sup> cells obtained from said individual with a chemokine compound, wherein the chemokine compound comprises a member selected from the group consisting of:

(i) at least one chemokine selected from the group consisting of MDC, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin; and

(ii) at least one chemokine of (i) comprising one or more conservative substitution, terminal additions and/or terminal deletions; and

(b) comparing the ability to isolate HIV from said cells with the ability to isolate HIV from a second aliquot of HIV<sup>+</sup> cells obtained from said individual that are not contacted with said chemokine compound;

(c) formulating the composition to comprise the chemokine compound which produces a decrease in the ability to isolate virus in the presence of said chemokine compound in the HIV<sup>+</sup> cells of the individual.

2. The method of claim 1, further comprising the step of combining in the composition two or more of said chemokines, demonstrating anti-viral activity against said HIV-1 isolates.

3. The method of claim 2 wherein at least 3 of said chemokines are combined.

4. The method of claim 1 further comprising repeating said contacting and comparing steps for at least 2 individual chemokines.

5. The method of claim 1 further comprising repeating said contacting and comparing steps for at least 3 individual chemokines.



7. The method of claim 1 wherein the HIV<sup>+</sup> cells are co-cultured with uninfected CD4<sup>+</sup> peripheral blood mononuclear cells prior to said contacting with the chemokines.

8. A method of formulating a pharmaceutical composition for a particular subject infected with HIV, the method comprising:

assaying at least one chemokine for the ability to inhibit:

HIV infection;

HIV replication; or

expression of an RNA or protein of HIV;

wherein said HIV is a primary isolate recovered from said subject; and

combining an amount effective of one or more of said chemokines demonstrating said ability with a pharmaceutically acceptable carrier to decrease the viral load in the isolate of said subject.

9. The method of claim 8 wherein said assaying of the chemokine is by a method comprising:

measuring HIV-1 levels in primary macrophage cells or primary CD4<sup>+</sup> peripheral blood mononuclear cells incubated with the primary isolate, which cells have been contacted with the chemokine; and

comparing the measured HIV-1 levels in the cells which have been contacted with the chemokine with said levels in cells not so contacted with the chemokine, wherein a lower level in said contacted cells indicates that the chemokine has anti-HIV activity.

10. The method of claim 9 wherein primary CD4<sup>+</sup> peripheral blood mononuclear cells are incubated with the primary isolate.

11. The method of claim 9 wherein the primary isolate has been propagated and maintained only in macrophages.

12. The method of claim 9 wherein the primary isolate is syncytia inducing.

13. The method of claim 9 wherein the primary isolate is non-syncytia inducing.



14. The method of claim 8 wherein said assaying of the chemokine is by a method comprising:
- measuring HIV-1 levels in cultures of HIV<sup>+</sup> cells obtained from the [patient] subject which have been contacted with the chemokine; and
- comparing said measured HIV-1 levels with said levels in said cells not so contacted with the chemokine, wherein a lower HIV-1 level in cultures of said contacted cells indicates that the chemokine has anti-HIV activity.
15. The method of claim 14 further comprising repeating steps (a) and (b) for at least 2 individual chemokines.
16. The method of claim 8 wherein the chemokine is a chemokine derivative and/or chemokine analog.
17. The method of claim 15 or 16 wherein the chemokine is selected from the group consisting of MDC, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin.
18. A method of inhibiting HIV infection or replication in cells of a subject in need of such treatment, the method comprising administering to the subject a pharmaceutical composition comprising:
- at least one chemokine selected from the group consisting of MDC, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin in an amount effective to inhibit HIV infection or replication in the cells of the subject; and
- a pharmaceutically acceptable carrier.
19. The method of claim 18 wherein the only chemokines in said composition are those demonstrated to have activity against a primary HIV isolate from said subject.
20. The method of claim 18 wherein said pharmaceutical composition comprises at least 2 of said chemokines.
22. A method of decreasing a HIV viral load in a subject in need of such treatment, the method comprising administering to the subject a pharmaceutical composition comprising:



at least one nucleic acid encoding a chemokine selected from the group consisting of MDC, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin, in an amount effective to decrease the HIV viral load; and

a pharmaceutically acceptable carrier.

23. The method of claim 22 wherein said composition comprises nucleic acids encoding at least 2 of said chemokines.

28. The method of claim 22 further comprising administering to the subject an anti-viral drug other than a chemokine, in an amount effective to inhibit HIV infection or replication.

29. The method of claim 28 wherein the other anti-viral drug is selected from one or more of the group consisting of AZT, ddI, ddC, 3TC, and zidovudine.

30. The method of claim 28 wherein the protein is administered intramuscularly.

37. A method of inhibiting HIV infection or replication in a subject in need of such treatment, the method comprising administering to the subject a composition comprising:

a first nucleic acid encoding RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$ , or IL-8, and

a second nucleic acid encoding a chemokine selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, lymphotactin and SDF-1;

together in an amount effective to inhibit HIV infection or replication.

38. A pharmaceutical composition comprising:

at least one chemokine selected from the group consisting of MDC, MCP-2, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin, in an amount effective to decrease a HIV viral load in infected cells; and

a pharmaceutically acceptable carrier.



39. The pharmaceutical composition of claim 38 wherein the chemokine is purified.
40. The pharmaceutical composition of claim 38 further comprising at least 1, 2, 3, 4, 5, 6, 8, or 9 chemokines in an amount effective to inhibit HIV infection or replication.
41. The pharmaceutical composition of claim 38 further comprising at least one member selected from the group consisting of RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$ , MCP-1, MCP-3, IL-8 or SDF-1 together in an amount effective to inhibit HIV infection or replication.
42. The pharmaceutical composition of claim 41 wherein the chemokine is a chemokine derivative and/or chemokine analog.
49. A pharmaceutical composition comprising:
- two or more chemokines, each of which binds to at least one chemokine receptor selected from the group consisting of CC CKR-1, CC CKR-2A, CC CKR-2B, CC CKR-3, CC CKR-4, CC CKR-5, CxCR4, IL-8RA, IL-8RB, Mig receptor,  $\gamma$ IP-10 receptor and Duffy antigen, in an amount effective to inhibit HIV infection or replication in infected cells; and
- a pharmaceutically acceptable carrier.
50. A method of formulating a pharmaceutical composition having anti-HIV activity against one or more HIV-1 isolates present in an individual at a given time, the method comprising:
- contacting a first aliquot of CD4<sup>+</sup> cells, one or more virus isolates obtained from said individual, and a chemokine; and
- comparing the ability to isolate HIV from said cells with the ability to isolate HIV from a second aliquot of CD4<sup>+</sup> cells contacted with said virus isolates that are not contacted with said chemokines,
- wherein a decrease in the ability to isolate virus in the presence of said chemokines is indicative that the chemokines has anti-viral activity against said HIV-1 isolates.
51. A pharmaceutical composition comprising MDC and I-309 in an effective amount to exhibit anti-HIV activity in human cells; and a pharmaceutically acceptable carrier.



54. The method of claim 18 wherein the MDC and I-309 are administered in a synergistically effective and therapeutically effective amount.